UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/576,633	11/14/2006	Shite Sebastian	14964	4672

25570 7590 06/17/2010

ROBERTS MLOTKOWSKI SAFRAN & COLE, P.C.
Intellectual Property Department
P.O. Box 10064
MCLEAN, VA 22102-8064

EXAMINER	
OGUNBIYI, OLUWATOSIN A	

ART UNIT	PAPER NUMBER
1645	

NOTIFICATION DATE	DELIVERY MODE
06/17/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

lgallaugher@rmsclaw.com
dbeltran@rmsclaw.com
docketing@rmsclaw.com

Office Action Summary	Application No.	Applicant(s)	
	10/576,633	SEBASTIAN ET AL.	
	Examiner	Art Unit	
	OLUWATOSIN OGUNBIYI	1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *08 March 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2 and 4-12* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-2 and 4-12* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

RESPONSE TO AMENDMENT

1. The amendment filed 3/8/10 has been entered into the record. Claims 1, 2 and 6 have been amended. Claims 3, and 13-22 are canceled. Claims 1-2 and 4-12 are pending in the application.

2. The species of the invention under examination is drawn to *S. aureus*, fluorescent materials, SEQ ID NO: 1 and wound dressing. See restriction requirement mailed 7/7/08 and the response to the restriction requirement filed 8/7/08.

However, in view of the instant amendment to claim 1 to recite "variants, homologs or fragments", the species requirement for the peptides is WITHDRAWN. The invention drawn to SEQ ID NO: 1-5 and variants, homologs or fragments are examined together.

Rejections Withdrawn

3. The rejection of claim 3 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement is withdrawn in view of the cancellation of the claim.

Rejections Maintained

4. The rejection of claims 1-2 and 4-12 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement is maintained for the reasons below. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. **This is an enablement rejection**.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, the amount of direction provided by the inventor, the existence of working examples, state of the prior art, the

level of predictability in the art and the quantity of experimentation needed to make or use the invention based on the content of the disclosure. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention and the breadth of the claims

The nature of the invention is the detection of a wound specific bacterium in a sample from a wound, a body fluid, or fluid from a wound by contacting a sample with a surface attached, detectably labeled peptide substrate selected from SEQ ID NO: 1-5 or variants or homologs or fragments of any SEQ ID NO: 1-5 under conditions that result in cleavage of said substrate by any protease enzyme produced in said sample by a wound specific bacterium; and detecting a cleavage or an absence of the cleavage of the substrate, the cleavage of the substrate indicating the presence of the wound-specific bacterium in the sample and the absence of the cleavage of the substrate indicating the absence of the wound specific bacterium in the sample.

The amount of direction provided and the existence of working examples

The specification contemplates the detection of the presence or absence of wound specific bacteria in a wound surfaces and body fluids (see p. 11 last bridging paragraph to p. 12 lines 1-10).

Example 2 p. 18-19, in the specification teach that wound dressings obtained from patients were extracted in PBS overnight and cleavage reaction was carried out on these samples with an RSL domain peptides e.g. SEQ ID NO: 1 and 2. Figures 10A-D present the results from this assay and the figure legend states that the graphs illustrate the relative fluorescence of bacteria extracted from wound dressings.

However, no information on the wounds or patients was obtained and thus it was not known that the wounds were infected with bacteria and the samples from the wound dressings were not cultured to determine whether or not the wound dressings were infected with bacteria in order to confirm the results of the cleavage assay.

The state of the prior art and the level of predictability in the art

The instantly claimed method does not take into account that wound surfaces and body fluids comprise human proteases (Steffensen et al Crit Rev Oral Biol Med 12(5):373-398, 2001, Armstrong et al J Am Podiatr Med Assoc 92(1): 12-18, 1998 and Ungar et al J Exp Med. 1961 January 31; 113(2): 359–380) that may be confounding factors in the instant method of bacterium detection. The specification in fig. 1A teaches that metalloproteinases (MMP1, MMP8 etc) from bacteria such as *S. aureus* cleave the RSL peptide of alpha-1-proteinase inhibitor. However, host matrix metalloproteinases such as MMP8 play a role in wound healing and can be found in wound tissue. Even bacterial infection of a wound results in prolonged elevation of proinflammatory cytokines which in turn causes increases in levels of matrix metalloproteinases released from neutrophils and macrophages (Cullen et al WO 03/040406 A2, 2003, p. 1 lines 24-28, p. 3 lines 8-15).

The instant method as claimed does not control for cleavage of the instant RSL domain peptides by non-bacterial enzymes or proteases are present in wound and body fluids and fluid from wounds because host enzymes similar to the same type of bacterial enzymes that cleave the instant peptides is also present in wound surface and the instant peptides comprises the same cleavage sites as is present in human alpha-1 proteinase inhibitor. For example, since host matrix metalloproteinases e.g. MMP8, are present in wound tissue, detection of cleavage of the instant peptides by these host enzymes will not correctly indicate that bacteria is present in said wounds. Desrochers et al (J. Clin. Invest. 1991 88:2258-2265, see whole document especially fig.5) teach that human MMP1 cleaves the human RSL domain of alpha-1-proteinase inhibitor and it is noted that instant peptide SEQ ID NO: 4 is present in alpha-1 proteinase inhibitor of humans. See figure 1B of the instant application. The instantly claimed method does not distinguish between modification of instant RSL domain peptides by proteases produced bacteria and by host. Since wound surfaces contain host enzymes that can cleave the detectably labeled RSL domain peptide it is not certain that the fluorescence observed is due to proteases produced by wound specific bacteria. Desrochers

teaches that matrix metalloproteinase 1 (MMP1) is synthesized in epithelial cells which can be found in wounds in response to proinflammatory cytokines (p. 2258 column 2) which in turn can be induced by the presence of bacteria (See. Xue et al Clinical and Experimental Ophthalmology vol. 28 issue 3 p. 197-200, 12/25/01).

Evidence provided by Applicants in the proposed publication filed along with the declaration filed 9/8/09 discloses the following in support of the fact that host proteases can also cleave the instant peptide substrates and thus does not distinguish cleavage by wound specific bacterium versus cleavage by host protease present in wounds:

> The ability of host proteases to process CPI2 (SEQ ID NO: 2) :
>
> *A number of research studies have demonstrated that the concentrations of neutrophil proteases such as human neutrophil elastase (HNE) and gelatinase-type matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) are elevated in chronic wounds compared to acute wounds (37-38). Since HNE is the primary target of α1-AP in vivo and both HNE and MMP-2 and -9 are known to cleave the reactive site loop of native α1-AP (32-34), we investigated the ability of these proteases to clip the CPI2 peptide. Commercial preparations of HNE or activated MMP-2 or MMP-9 were diluted to physiologically elevated levels and analyzed in the protease assays. The increase in Vmax signal seen with HNE demonstrated that this protease was able to efficiently clip CPI2 comparable to the V8 bacterial protease control (Figure 7).*

Thus, in the case whereby a wound or body fluid including wound fluids are not infected with bacteria or the wound or body fluid including wound fluids are infected with bacteria that do not produce the right protease, cleavage of the instant peptides by host proteases would interfere with the instant method and would not result in detection of the presence or absence of a wound specific bacterium.

In view of the above, undue experimentation would be required of the skilled artisan to practice the invention as claimed.

Applicants' arguments and the response.

Applicants argue that the declaration of Dr. Mitchell C. Sanders under 37 CFR 1.132 filed 9/8/09 and the accompanied proposed publication and poster demonstrates the bacterium detection method of the claims as amended herein and is highly effective at detecting pathogenic bacteria present in wounds and wound fluids.

Applicants' argument is carefully considered but is not persuasive. The declaration under 37 CFR 1.132 filed 9/8/09 is insufficient to overcome the instant rejection as set forth above.

The declaration states that the detection method of claims 1-12 as amended relies on the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 μM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag). A dressing sensor consisting of a polyurethane foam bottom layer and the CPI2S RSL peptide-blue bead chemistry at the top membrane is also depicted in the poster submitted with the instant declaration.

Applicants' statement that the "the detection method of claims 1-12 as amended relies on the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 μM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag)" is not commensurate with the scope of the claims. The instant claims do not teach that the peptides are placed between an anchor and an affinity tag as stated by Applicants. See declaration p. 3.

The declaration further states that the steric hindrance of labeling a small peptide with very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus makes it virtually inaccessible to host proteases that are typically much larger than 30 K.

Applicants' arguments on p. 7 of the reply filed 3/8/10 states that "It's applicants' position that the information provided by the Sanders Rule 132 Declaration and in the accompanying proposed publication and poster demonstrates that the bacterium

detection method of the claims herein is highly effective at detecting pathogenic bacteria present in wounds or wounds fluids. This is carefully considered but is not persuasive.

As stated above, the Sanders declaration states that "the detection method of claims 1-12 as amended relies on the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 µM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag)". The poster submitted also is drawn to the peptide placed between a bead of particular size and a label which is attached to a dressing consisting of a polyurethane foam bottom layer and the CPI2S RSL peptide-blue bead chemistry. The proposed publication submitted as part of the declaration filed 9/8/09 is also drawn to the labeled peptide conjugated to beads of a particular size. Thus, the bacterium detection method of the declaration, the poster and the proposed publication is not commensurate with the scope of the instant claims. The Sanders declaration states that the detection method of claims 1-12 as amended relies on the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 µM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag)" , this is not found in the claims.

Furthermore, Applicants statement that the Rule 132 declaration demonstrates that the peptide substrates used are not significantly cleaved by any host enzymes such as MMPs is not persuasive. The data in figure 1 is generated using the peptide CPI2 (SEQ ID NO: 2) conjugated to said bead, which is not found in the claims and the importance of the bead as stated by the declaration is that the steric hindrance of labeling a small peptide with very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus makes it virtually inaccessible to host proteases that are typically much larger than 30 K and in contrast most MMPs are 30-125 kDa. See p. 3 of the declaration.

Furthermore, the scope of claim 1 and dependent claims includes other peptides SEQ ID NO: 1 and 3-5 as well as homologs or variants or fragments thereof as wells as homologs or variants or fragments of SEQ ID NO: 2, while the data provided in fig. 1 of the declaration is only drawn to experiments done with SEQ ID NO: 2 anchored between a bead and label.

Furthermore, Applicants argument on p. 8 state that *in vitro* data provided that the *anchored* substrates used do not cross react with MMPs 1, 2,9, 12 and 13. This is not found persuasive. Again, the instant claims do not recite that the peptide substrates are anchored to beads and the Sanders declaration states that the detection method of claims 1-12 as amended relies on the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 µM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag)" , this is not found in the claims. Also, the declaration is only drawn to data provided that the bead anchored SEQ ID NO: 2 which does not cross react with MMPs 1, 2 and 9, but does not provide any evidence for MMPs 12 and 13.

Applicants admit that human neutrophil elastase (HNE) reacts with peptides such as CPI2 and that there is clinical evidence to suggest that HNE is normally only active when there is a high bacterial load to activate the pro-enzyme. This is carefully considered but is not found persuasive. The instant claims do not specify the bacterial load in the wound or body fluid or fluid from a wound and Applicants do not provide the clinical evidence relied upon.

The proposed publication titled "Rapid Measurement of Protease Activity Prevalent in Bacteria from Wounds: A diagnostic for Total Bioburdern: submitted along with the instant declaration teach a peptide named "CPI2S" which has the sequence GMAFLEAIPC (see p. 5 of 46) which is different from the claimed sequence of CPI2 (SEQ ID NO: 2 which has the sequence EGAMFLEAIPMSIPK). The publication states that the CPI2S peptide attached to a bead has reduced sensitivity to human neutrophil

elastase (p. 14 of 46 first paragraph, p. 19 of 46 second paragraph, figure 7 legend p. 36 of 46). However, the CPI2 (SEQ ID NO: 2) peptide attached to bead was clipped by human neutrophil elastase. This "CPI2S" peptide having the sequence GMAFLEAIPC is not disclosed in the claims or the specification.

The evidence as set forth above in the declaration is not commensurate with the scope of the claims because the evidence in the declaration is drawn to the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 μM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag).

The claims do not disclose a 10 amino acid peptide and the claims do not indicate that the peptide is conjugated to a bead of a particular size and that the label is a blue dye #1 or biotin/polyhistidine tag. The Sanders declaration clearly states that the detection method of claims 1-12 as amended relies on the use of small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 μM Tris Acryl or HyperD bead) and an affinity tag (for example, either blue dye#1 or biotin or polyhistidine dual affinity tag)". It is the steric hindrance of labeling the small peptide with a very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus that makes it virtually impossible for host proteases to cleave the RSL small peptide.

This structure of a 10 amino acid peptide anchored to a very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus is not recited in the claims.

Applicants argues that the Examiner asserts, for example, that the Rule 132 declaration shows operability only of methods using alpha-1-peptide substrates anchored to a certain type of beads and certain type of aromatic markers and that all three elements are appropriately representative of the types of materials which can be used. This is not found persuasive in view of Applicants statements in the declaration.

Applicants/ declaration states that:

> *The detection method of Claims 1-12 as amended herein, relies on the use of a small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor that is placed between an anchor (for example, a 60 µm Tris Acryl or HyperD bead, Pall Life Sciences) and an affinity tag (for example, either blue dye #1 or biotin/poyhistidine dual affinity tag). The steric hindrance of labeling small peptide with very large bead on the amino terminus and a bulky ring structure of dye or biotin molecule on the carboxy terminus makes it virtually inaccessible to host proteases that are typically much larger (30 kDa) than the active proteolytic form of a bacterial protease. Most active forms of bacterial proteases are 30 kDa, in contrast most MMPs are 30-125 kDa and are often found as dimeric complexes.*

All the evidence of provided in the declaration, in the poster, in the proposed declaration are drawn to the peptide conjugated to beads. Furthermore, the poster is drawn to the peptide CPIS which is not disclosed in the instant claims. In addition, the claims do not disclose a 10 amino acid substrate as stated in the declaration (i.e. "*The detection method of Claims 1-12 as amended herein, relies on the use of a small (10 amino acid) region of the reactive site loop (RSL) of alpha 1 trypsin inhibitor*"). (Furthermore, the proposed publications stated that the CPI2 (SEQ ID NO: 2) peptide attached to bead was clipped by human neutrophil elastase which is a host protein found in wounds.

Page 20-21 of the proposed publication includes the following paragraphs"

> <u>The ability of host proteases to process CPI2 :</u>
>
> *A number of research studies have demonstrated that the concentrations of neutrophil proteases such as human neutrophil elastase (HNE) and gelatinase-type matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9) are elevated in chronic wounds compared to acute wounds (37-38).* Since HNE is the primary target of α1-AP *in vivo* and both HNE and MMP-2 and -9 are known to cleave the reactive site loop of native α1-AP (32-34), we investigated the ability of these proteases to clip the CPI2 peptide. Commercial preparations of HNE or activated MMP-2 or MMP-9 were

diluted to physiologically elevated levels and analyzed in the protease assays. The increase in Vmax signal seen with HNE demonstrated that this protease was able to efficiently clip CPI2 comparable to the V8 bacterial protease control (Figure 7). In contrast, the signal for HNE with the shorter version CPI2S produced a signal that was much reduced without affecting the activity of V8 protease. No significant activity was seen with MMP-2 or MMP-9 with either peptide suggesting that none of the residues in CPI2 are sensitive to MMPs (not shown).

In Table 1, a direct comparison of the amount of HRP released from CPI2S and CPI2 HRP-beads by bacterial proteases present in CFGM shows that the bacterial proteolytic activities are retained while dramatically reducing the sensitivity to HNE. In particular, proteases from *P. aeruginosa* or *S. pyogenes* released similar amounts of HRP from CPI2 and CPI2S HRP-microbeads. S. *aureus* and *E. faecalis* proteases were more active toward HRP-CPI2- microbeads than HRP- CPI2S-microbeads, but the ratio of activity from bacterial proteases and HNE showed that CPI2S appears much more selective for bacterial proteases than CPI2. The bacterial protease-to-HNE activity ratios reflect that CPI2S is much less sensitive to HNE relative to each bacterial protease. Thus the reduced ability of HNE to clip CPI2S was not accompanied by a corresponding reduction in bacterial protease activity. This truncation of the peptide substrate therefore improved the specificity of detection of bacterial proteases.

Table 1 of the proposed publication on p. 33:

Table 1: Comparison of protease cleavage of CPI2S and CPI2

	# of Strains with Activity	CPI2 Activity Vmax (OD_{405nm})	CPI2S Activity Vmax (OD_{405nm})	bact/HNE ratio CPI2	bact/HNE ratio CPI2S
HNE	NA	106 +/- 6.6	6.9 +/- 1.8	n/a	n/a
S. aureus	18/20 (90%)	188 +/- 20	82.9 +/- 5.0	1.8	12.1
E. faecalis	16/20 (80%)	80.7 +/- 7.5	44.9 +/- 0.9	0.8	6.5
S. pyogenes	17/20 (85%)	25.7 +/- 2.5	18.8 +/- 0.9	0.2	2.7
P. aeruginosa	18/19 (94.7%)	194 +/-13.4	152 +/- 31.1	1.8	22.2

Clearly, physiological levels of HNE a human host protein is able to cleave both the CPI2 (SEQ ID NO: 2) and CPI2S peptides conjugated to beads albeit CPI2s at lower levels.

Figure 7 legend of the proposed publication on p. 36 states:

Figure 7: ***Improvement of substrate peptide to reduce cleavage by host proteases.*** *Biotin-polyhistidine CPI2 peptide (circles) on beads shows a concentration dependent increase in signal from human neutrophil elastase (HNE) tested in the 0-1 µg/ml range. The peptide on beads was exposed to HNE concentrations for 5 minutes and then the beads were filtered out of solution. The released tag was measured by ELISA and TMB color development. The graph shows the Vmax at 650 nm for each concentration. The HNE cleavage site was removed from the CPI2 peptide in a newer version of the substrate: CPI2S. CPI2S shows reduced HNE activity (triangles) tested in the same manner in the same concentrati0n range. Also shown is the reactivity of CPI2 and CPI2S peptide to purified V8 bacterial protease (diamonds and squares respectively) in the 0-1 µg/ml range which is not greatly affected by the removal of the elastase cleavage site. MMP 2 and MMP 9 (0.5 µg /ml) were also tested on the CPI2 peptide. The results (not shown) yielded the same signal as a control indicating no CPI2 cleavage.*

In conclusion, the totality of evidence submitted by Applicants is drawn to the peptide substrates attached to beads and the evidence is drawn to the CPI2 peptide attached to beads and is not commensurate with the scope of the claims which recites other peptide substrates apart from CPI2 and the claims do not set forth the peptide is anchored to beads that makes it virtually inaccessible to host proteases. Furthermore, the evidence shown in the proposed publication in table one shows that for the CPI2 (SEQ ID NO: 2) or CPI2S peptide conjugated to bead is still cleaved by physiological levels of host HNE enzyme albeit at lower levels for the CPIS peptide.

As to Applicants argument that "CPI2S" which has the sequence GMAFLEAIPC is an obvious variant of CPI2 substrate and such fragment is described and expressly claimed in the application as being suitable and workable alternatives to the specific substrates which have their longer amino acid sequences set forth. This is carefully

considered but is not found persuasive. "CPI2S" which has the sequence GMAFLEAIPC is not expressly claimed. The claims do not claim a peptide consisting of the sequence GMAFLEAIPC and the recitation of variants, homologs or fragments thereof is not an a substitute for express disclosure of a peptide consisting of the sequence GMAFLEAIPC in the specification or in the claims.

In fact inclusion of the sequence GMAFLEAIPC in the claims would constitute new matter and would not comply with the written description prong of 35 USC 112 1st paragraph.

In Purdue Pharma L.P. v. Faulding Inc., 230 F.3d 1320, 1326, 56 USPQ2d 1481, 1486 (Fed. Cir. 2000), the court noted that with respect to In re Ruschig 379 F.2d 990, 154 USPQ 118 (CCPA 1967) that "Ruschig makes clear that one cannot disclose a forest in the original application, and then later pick a tree out of the forest and say "here is my invention". In order to satisfy the written description requirement, the blaze marks directing the skilled artisan to that tree must be in the originally filed disclosure." In the instant case, the description of the peptide substrates SEQ ID NO: 1-5 does not point one of ordinary skill in the art to the peptide GMAFLEAIPC (CPIS) which has the biochemical property of being able to be cleaved by a protease produced by a wound specific bacterium. There are no 'blaze marks" in the specification as filed directing the skilled artisan to this peptide. There is no direction in the specification directing one of skill in the art to pick this peptide out of all the possible variants of SEQ ID NO: 1-5. The introduction of claim changes which involve narrowing the claims by introducing elements or limitations which are not supported by the as-filed disclosure is a violation of the written description requirement of 35 U.S.C. 112, first paragraph. The original disclosure of a large genus did not support a later filed claim to a previously unnamed single species. See In re Ruschig, 371 F.2d 990, 154 USPQ 118 (CCPA 1967). This is would constitute new matter because the specification as filed did not provide written description support for the skilled artisan to pick GMAFLEAIPC (CPIS) that can be

cleaved by a protease produced by a wound specific bacterium out of all the plethora of variants of SEQ ID NO: 1-5.

New Rejections Based on Amendment

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

5. Claims 1-2 and 4-12 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a written description rejection.**

The claims are drawn to a method for detecting the presence or absence of a wound-specific bacterium in a sample selected from a wound, a body fluid or fluid from a wound, said method comprising the steps of:

a) contacting said sample with a surface-attached, detectably labeled synthetic al-proteinase inhibitor reactive site loop domain peptide substrate selected from the group consisting of EAAGAMFLEAIPK (SEQ ID NO: 1), EGAMFLEAIPMSIPK (SEQ ID NO: 2), KGTEAAGAMFLEAIPMSIPPEVK (SEQ ID NO: 3), GAMFLEAIPMSIPPE (SEQ ID NO: 4), CGAMFLEAIPMSIPAAAHHHHH (SEQ ID NO: 5), and variants, homologs or fragments of any of said peptide substrates, under conditions that result in cleavage of said substrate by an a protease enzyme produced in said sample by a wound-specific bacterium; and

b) detecting a cleavage or an absence of the cleavage of the substrate, the cleavage of the substrate indicating the presence of the wound-specific bacterium in the sample and absence of the cleavage of the substrate indicating absence of the wound-specific bacterium in the sample.

The claims encompass the use of variants, homologs or fragments of any of SEQ ID NO: 1-5 in the instant method of detecting the presence or absence of a wound specific bacterium in a sample.

Page 14-16 of the specification discloses the scope of variants, homologs or fragments as follows:

Variants include a substantially homologous polypeptide encoded by the same genetic locus as these peptide substrates e.g. the α1-RSL domain in an organism, i.e. an allelic variant as well as other variants.

Variants also encompass polypeptides derived from other genetic loci in an organism, but having substantial homology to a peptide substrate described herein. Variants also include polypeptides substantially homologous or identical to these peptide substrates, but derived from another organism, *i.e., an* ortholog. Variants also include polypeptides that are substantially homologous or identical to these peptide substrates, that are produced by chemical synthesis. Variants also include polypeptides that are substantially homologous or identical to these peptide substrates, that are produced by recombinant methods.

The invention also encompasses polypeptide substrates having a lower degree of identity but having sufficient similarity so as to perform one or more of the same functions performed by the peptide substrate, *e.g.,* the ability to act as a substrate for enzymes produced or secreted by bacteria, for example, wound-specific bacteria. Similarity is determined by conserved amino acid substitution. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics. Conservative substitutions are likely to be phenotypically silent. Typically seen as conservative substitutions are the replacements, one for another, among the aliphatic amino acids Ala, Val, Leu, and Ile; interchange of the hydroxyl residues Ser and Thr; exchange of the acidic residues Asp and Gin; substitution between the amide residues Ash and Gin; exchange of the basic residues Lys and Arg; and replacements among the aromatic

residues Phe and Tyr.

Functional variants can also contain substitution of amino acids similar to those in the or1 RSL domain that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree. Non-functional variants typically contain one or more non- conservative amino acid substitutions, deletions, insertions, inversions, or truncation or a substitution, insertion, inversion, or deletion in a critical residue or critical region, such critical regions include the proteolytic cleavage site for an infection-specific protease.

Amino acids in a peptide substrate of the present invention that are essential for cleavage by an enzyme, e.g., a protease, can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham et al , Science, 244:1081-1085 (1989), incorporated herein by reference). The latter procedure introduces a single alanine mutation at each of the residues in the molecule (one mutation per molecule).

The invention also includes polypeptide fragments of the peptide substrates or functional variants thereof, including biologically active fragments with 60%, 70%, 80%, 90% or 95% sequence homology to a synthetic or naturally-occurring peptide substrate described herein, e.g., the al RSL domain sequence. The present invention also encompasses fragments of the Variants of the polypeptides described herein. Biologically active fragments include fragments that have retain the ability to act as substrates for enzymes produced or secreted by bacteria, for example, wound-specific bacteria. Fragments can be discrete (not fused to other amino acids or polypeptides) or can be within a larger polypeptide. Further, several fragments can be comprised within a single larger polypeptide. In one embodiment a fragment designed for expression in a host can have heterologous pre- and pro-polypeptide regions fused to the amino terminus of the polypeptide fragment and an additional region fused to the carboxyl terminus of the fragment.

Therefore, the instant method encompass the use of a large genus of structurally distinct variants (including homologs or fragments) of each of SEQ ID NO: 1-5 for detecting the presence or absence of a wound specific bacterium.

The specification discloses the reduction to practice of detecting the presence or absence of bacteria in wound dressings, see example 2 p. 18-19. The wound dressings obtained from patients (no information on the wounds or patients was obtained) were

extracted in PBS overnight and cleavage reaction was carried out on these samples with an RSL domain peptide of alpha-1-proteinase inhibitor i.e. a CPI2 peptide substrate (SEQ ID NO: 2). Figures 10A-D present the results from this assay and the figure legend states that the graphs illustrate the relative fluorescence of bacteria extracted from the wound dressings.

Although, the specification does not reduce to practice detection the presence or absence of wound specific bacteria in a sample such as wound, a body fluid or fluid from a wound using SEQ ID NO: 1, the specification teach that SEQ ID NO: 1 labeled or SEQ ID NO: 2 labeled or SEQ ID NO: 5 labeled used as the substrate was contacted with bacterial culture and protease activity was observed for a number of the bacteria with the peptide substrates (in the case of SEQ ID NO: 5 cleaved with a protease from *Pseudomonas aeruginosa*). See example 3 and example 4. SEQ ID NO: 4 is the alpha-1-proteinase inhibitor RSL sequence and SEQ ID NO:3 is the human alpha-1-proteinase inhibitor. See figures 1A and 1B and the p. 5 lines 9-13.

There is no description of variants or homologs or fragments of each of SEQ ID NO: 1-5 that can be used as substrates which under conditions that result in cleavage of said substrate by an a protease enzyme produced in said sample by a wound-specific bacterium would result in the detection of the presence or absence of said wound-specific bacterium.

There is no disclosure of the common structure of the genus of homologs, variants or fragments encompassed by the specification as set forth above that can be cleaved by any wound-specific bacterium result and result in the detection of the presence or absence of said wound-specific bacterium.

For each protein i.e. each of SEQ ID NO: 1-5, the genus of homologs, variants or fragments encompassed by the specification is highly variant, and the specification does not describe the common structure of the genus of homologs, variants or fragments of each of SEQ ID NO: 1-5 that would result in a substrate that can be cleaved by any

protease produced in the recited samples by a wound-specific bacterium and result in the detection of the presence or absence of said wound-specific bacterium.

The disclosure of each of the proteins i.e. SEQ ID NO: 1-5 is insufficient to describe the genus of each protein that can used as a substrate for proteases produced by wound specific bacteria in the recited samples.

It is well known in the art that amino acid substitutions, deletions and insertions in a protein's sequence can affect the protein's structure and thus its function. See review articles: Bowie et al. Science Vol. 247, No. 4948, p. 1306-1310,1990; Lazar et al. Molecular and Cellular Biology, Mar. 1988, p. 1247-1252 and Burgess et al. The Journal of Cell Biology, vol. 111, Nov. 1990 2129-2138. Consequently, the effects of sequence dissimilarities upon protein structure and function cannot be predicted. Bowie et al (Science, 1990, 257:1306-1310) teach that an amino acid sequence encodes a message that determines the shape and function of a protein and that it is the ability of these proteins to fold into unique three-dimensional structures that allows them to function and carry out the instructions of the genome and further teaches that the problem of predicting protein structure from sequence data and in turn utilizing predicted structural determinations to ascertain functional aspects of the protein is extremely complex (column 1, page 1306). Bowie et al further teach that while it is known that many amino acid substitutions are possible in any given protein, the position within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of maintaining function are limited. Certain positions in the sequence are critical to the three dimensional structure/function relationship and these regions can tolerate only conservative substitutions or no substitutions (column 2, page 1306). Presently, genus of variants, homologs and fragments of each of SEQ ID NO: 1-5 is large and highly variable and it is unpredictable as to which ones would cleaved by a protease produced by a wound specific bacterium.

In such an unpredictable art, as set forth supra, adequate written description of a genus (e.g. the genus of homologs, variants or fragments thereof of each of SEQ ID

NO: 1-5) which embraces widely variant species cannot be achieved by disclosing only one species within the genus (i.e. each of SEQ ID NO: 1-5). See *Noelle v Lederman*. 355 F. 3d 1343, 1350, 69 USPQ2d 1508, 1514 (*Fed. Cir. 2004*) and *In re Alonso* (Fed. Cir. 2008-1079). The description of more species with the instant activity will be required where the claimed genus is highly variable.

The written description requirement is separate and distinct from the enablement requirement (See also *Univ. of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 920-23, 69 USPQ2d 1886, 1890-93 (Fed. Cir. 2004) and adequate written description requires more than a mere reference to a potential method for identifying candidate homologs, variants and fragments thereof of each of SEQ ID NO: 1-5. The purpose of the written description requirement is broader than to merely explain how to 'make and use' [the invention] *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1114 (Fed. Cir. 1991).

One of ordinary skill in the art as of the time of filing, would not have been able to envision homologs (including orthologs), variants (including insertion, substitution or deletion variants) or fragments of each of SEQ ID NO: 1-5 that would result in cleavage of said homologs, variants or fragments by a protease enzyme produced by a wound specific bacterium in wound, body fluid or fluid from a wound and result in detecting the absence or presence of a wound-specific bacterium in wound, body fluid or fluid from a wound.

Applicants as of the time of filing, were only in possession using SEQ ID NO: 1-5 which can be cleaved by protease enzyme produced by wound specific bacterium.

For further guidance, Applicants are directed to the Final Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001 and revision of the Written Description Training materials, Revision 1 March, 2008 http://www.USPTO.gov/web/menu/written.pdf.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claims 1-2, 4-6 and 8-12 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 and dependent claims are drawn to "a method for detecting the presence or absence of a wound-specific bacterium in a sample selected from a wound, a body fluid or fluid from a wound…".

Since the claims are drawn to detecting the presence or absence of a wound-specific bacterium, it is not clear how the presence of a wound specific bacterium is detected in body fluids other than wound-fluids. It is not clear whether the claims are only limited to detecting the presence or absence of bacteria found only in wounds. Please clarify the metes and bounds of the claims.

Status of Claims

Claims 1-2 and 4-12 are rejected. No claims allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not

mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to OLUWATOSIN OGUNBIYI whose telephone number is 571-272-9939. The examiner can normally be reached on M-F 8:30 am- 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Robert Mondesi can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Oluwatosin Ogunbiyi/
Examiner, Art Unit 1645

/Robert B Mondesi/
Supervisory Patent Examiner, Art Unit 1645